مجلة كلية التربية الاساسية
كلية التربية الاساسية – الجامعة المستنصرية
P- ISSN: 3007-3650 E- ISSN: 2706-8536
Journal of the College of Basic Education Vol.32 (No.137) 2026,pp.971-995

Evaluation of the *rmpA* Gene as a Virulence Factor Regulating Biofilm Formation in *Klebsiella pneumoniae*.

Hawraa Qasim Owaid[1] , Maysoom Kh Abbas[2]
[1,2]Department of Biology, College of Science, Al-Mustansiriyah University, Baghdad, Iraq.
Corresponding Email :- hawra98@uomustansiriyah.edu.iq
maysoon.bio2005@uomustansiriyah.edu.iq,

Abstract

Klebsiella pneumoniae has emerged as a significant pathogen globally, particularly in clinical settings, resulting in severe infections, including UTI, respiratory tract infections, and bloodstream infections, and the high rates of resistance to the most common drugs make it very difficult to treat. Numerous characteristics that increase virulence are responsible for the observed pathogenesis of bacteria. This study aimed to investigate biofilm-forming ability among the collected isolates and the presence of the virulence gene *(rmpA)*. And to determine gene association with increased biofilm formation. Methods: seventy-five clinical isolates were gathered from different clinical samples (urine, blood, sputum, and stool). Antibiotic susceptibility was tested against a number of commonly used medications, including beta-lactam antibiotics. Biofilm formation was evaluated using the plate assay method, and the presence of the targeted gene was determined by molecular analysis. Result: Most isolates showed high resistance to beta-lactams, with nearly eighty percent of them being resistant. Among the highly effective drugs were carbapenems (DOREBENEM)with (82.7%). And for the biofilm development test, it was shown that a total of 86.6% of the *Klebsiella pneumoniae* isolates were biofilm producers, ranging from weak to strong, while 13.4% did not produce biofilm. Additionally, the molecular level revealed a variable distribution of the targeted gene in the study, which indicates the presence of a virulent gene (*rmpA*) (58.6%). In conclusion, it has been demonstrated that *Klebsiella pneumonia* isolates exhibit a strong biofilm-forming capacity increased the rates of resistance to antibiotics. And

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concerning the relation with the *rmpA* gene, a positive association was detected between biofilm formation and rmpA-carrying isolates, although it was strain dependent. This indicates that multiple factors (genetic, environmental), rather than a single gene, can control the intricate processes of biofilm development.

Keywords: *Klebsiella pneumonia*, biofilm, rmpA gene

1. Introduction

A naturally occurring member of the enteric bacterial group, *Klebsiella pneumoniae*, is part of the intestinal microbiome of healthy individuals. This prevalent opportunistic pathogen accounts for roughly ahigh rates of the infections that quietly occur, specially at healthcare regions. It is linked to potentially deadly infections like endocarditis and sepsis, as well as additional gastrointestinal infections like pneumoniae, cystitis, urinary tract infections, and surgical site infections. The World Health Organisation (WHO) listed this bacterium as one of the most dangerous infections in the world due to rising treatment resistance, high patient fatality rates, and challenges in treating the infection. [1]. In recent years, bacteria have become more significant infectious microorganisms due to the increasing spread, as well as the rise of resistance to the more commonly used antibiotics, which makes the treatment options difficult. The infection was commonly detected in hospitals and considered the second most common cause of Gram-negative bacteremia in individuals with weak immunity [2]. The pathogenicity of the bacterium is driven by multiple traits; these traits increase the bacterium's pathogenicity and allow it to escape the immune system, including a polysaccharide capsule that encases it. The lipopolysaccharides that cover the bacteria's outer surface cause inflammation. Another crucial virulence element is the bacterial cell's fimbriae, which enable the organism to adhere to host cells, and iron-chelating substances, such as siderophores released by bacterial cells, which allow the organism to proliferate by obtaining iron from the host. [3] One of the primary mechanisms of bacterial resistance is their ability to develop biofilm, posing a major problem in various industries(healthcare, food processing, water treatment), resulting in the

distribution of hard-to-control infections caused by the microorganisms. However, the bacteria frequently develop biofilms when they are exposed to external environmental stresses, oxidative stress, high osmotic pressure, low pH, severe nutritional excess or deficiency, antibiotics, and antimicrobial agents [4,5]. Prolonged, complex biofilm processes and the use of numerous drugs weaken the host's defenses, which contribute to the increased prevalence of k. Pneumonia. causes sickness. Since hospital infections are also known to harbor MDR isolates, grown to be a significant worry worldwide[6]. The complicated and persistent structure of biofilms causes a complete eradication challenge, and their association with the rise in rates of resistance to medications highlights the importance of a detailed study of such traits. Even though numerous approaches to biofilm contamination have been developed in recent decades, it remains difficult to manage. Antibiotic resistance is the most well-known feature of biofilms. The main factor that determines resistance is the growth condition of the bacteria. Because the bacteria in the "inner core" of a biofilm have adapted to starvation and low oxygen levels, they can stop growing and make antibiotics that target metabolically active and dividing bacteria less effective [7]. **The aim of the study is** to identify biofilm-producing isolates with increased antibiotic resistance, and to determine gene association with increased biofilm formation

2. Materials &methods

2.1 Identification of bacterial isolates

2. 1.1 Morphological Analysis (on culture and microscope)

During a period of two months, 75 Klebsiella pneumoniae clinical isolates were gathered from various sources (urine, wounds, burns, sputum, and stool). Over the course of two months, bacterial isolates were acquired from AL-Imam ALI Hospital, AL Kindy Teaching Hospital, and Baghdad Teaching Hospital (Medical City Hospital). By using a transport medium, each sample was labeled with the patient's information (sex, age, date, and sample type). The collected isolates were then inoculated on MacConkey agar in order to activate the isolates and

detect their phenotypic characteristics, followed by incubation at 37 °C for 24 hr. After that, several examinations were performed for accurate identification.

Ethics statement and consent to participate:

The authors confirm that the ethical policies of the journal are noted on the journal's author guidelines page. The study was approved by the Ethics Committee of the Department of Biology, College of Science, Al-Mustansiriyah University, Baghdad, Iraq (Approval Number: BCSMU/11025/00087M). College of Science, University of Al-Mustansiriyah.

2.1.2. Biochemical methods for bacterial identification

Biochemical testing methods were used, including the Lactose fermentation, the test of Triple Sugar Iron agar, the catalase test, the Citrate utilization test, oxidase, and hydrogen sulfide generation. The Analytical Profile Index System (API20E system) was used for the identification of the isolates. According to the French supplier's company guidelines (BioMérieux), the test was conducted. This analytical system was used for testing the remaining biochemical tests, as well as to certify the isolates' diagnosis as followed: At first, the strip was set up, into each well of the plastic tray distal water about five milliliters of sterile solution was added, to create a moisture environment, and this occurred before the strip was placed within the plastic tray. For the next step, the preparation of bacterial suspension was performed from newly cultured plates that were incubated for 24 hr at 37 C. And into a sterile tube harboring PBS (5ml), about 3 to 4 colonies were transferred and mixed by vortexing for a few seconds. Following the suspension preparation, strip inoculated into each micro capule with bacterial suspension using a sterile syringe, carefully into each micro capule.

2.2 Antibiotic sensitivity test

The procedure (Kirby-Bauer) is followed according to Brooks et al. (2007).In order to prepare a bacterial suspension, colonies from an overnight incubated culture (four to five colonies) were transferred and put into a tube containing roughly 3 to 5 mm of normal saline [8], using

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13 discs of drugs include, broad spectrum antibiotics such carbapenems (dorebenem, fluoroquinolones, Ciprofloxacin, penicillin G, amoxicillin, ampicillin) and cephalosporins (Cefepime, ceftazidime). (Table 1). The isolate was subjected to a susceptibility test using the Kirby-Bauer technique.

TABLE 1 shows the antibiotic discs and their source (origin)that were used in the study

No	ANTIBIOTIC	Code	Concentration Antibiotic (µg/disc)	Company origin
1	Ciprofloxacin	CIP	5µg/disc	Bioanalye (Turkey)
2	Oxacillin	OX	10µg/disc	
3	Rifampicin	RIF	5µg/disc	
4	Ofloxacin	OFX	5µg/disc	
5	Aztreonam	ATM	30µg/disc	
6	Amoxicillin	AX	10µg/disc	
7	Penicillin G	P	10µg/disc	
8	Vancomycin	VA	5µg/disc	
9	Doripenem	DOR	10µg/disc	
10	Gentamicin	GEN	10µg/disc	
11	Cefepime	CPM	30µg/disc	
12	Ampicillin/Sulbactam	Amp/S	10/10µg/disc	
13	Ceftazidime	CAZ	30µg/disc	

2.3. Biofilm development detection:

The microtiter plate assay was the method that was used for the identification of the generation of the biofilm. In accordance with the steps that were stated by Babapour et al. (2016):

•First, about three to five colonies that were newly cultivated in an agar plate were taken using a sterile loop, and then were transferred to a test tube that contains 10 ml of media (Trypticase soy broth), where, combined with Glucose solution (1%), the turbidity of bacterial suspension was adjusted to match (0.5 Mcfarland standard).

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•The tubes of bacterial suspention then were then incubated for 18-24 hr at 37 °C.
•After incubation,100ul of the suspension was added to 9900 ul of fresh Trypticase soy broth(TSB), in order to dilute it 1:100 with new medium.
•200 ul of the previously prepared solution (The diluted bacterial suspension) was added to each well of the sterile 96 flat-bottom polystyrene microtiter plate using a micropipette.
•Furthermore, to guarantee there is no contamination &detect the non-adherent cells to wells surface, only sterile broth was employed as a negative control. Each of the bacterial isolates was inoculated in triplicate to ensure accuracy, the plate was covered with its lid, and incubated for about 24 hr at 37 C.
•Following incubation, remove the plates and lightly tap or invert to extract the content of wells, then wash with 200 µL of phosphate buffer saline(pH 7.2), washing was done twice in order to exclude non-binding cells.The adhesive bacteria that formed biofilm in the wells of the plates were secured by incubation (for 30 min at 37 °C).
•Next, the wells were dyed using crystal violet solution (1%), 200 µL of the dye was added to each well for 45 min.
•After the staining step, the plate was washed 3 times by sterile distilled water to remove extra dye and then left to dry at room temperature.
•Adding 200 ul of ethanol to dissolve the dye that bound to adhering biofilm, and left for15 to 20 minutes.
•Finally, the optical densities of the stained bacterial isolates were measured using an ELISA reader set to a wavelength of 630nm. The values of the OD for biofilm detection were categorized according to (Mathur et al., 2006), into strong(biofilm producer), moderate, and non or weak (non-producer)

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2.4. Diagnostics of the target virulence gene

2.4.1. The process for extracting DNA from Klebsiella pneumoniae

Genomic DNA was extracted from seventy-five clinical isolates of *Klebsiella pneumoniae*, using a commercial purification system kit(Bioneer, South Korea), Table 2. DNA concentration and purity estimation were measured using a NanoDrop device, which was utilized to quantify DNA concentration and purity, at wavelength 260-280nm, which ranges from 1.8 to 2.0 [11].

Table 2: The content of the DNA extraction kit

COMPONENT	SUPPLIED AMOUNT
ELUTION BUFFER	30 ml
MINI SPIN COLUMNS	100 units
COLLECTION TUBES	200 units
USER MANUAL	1 copy
CONCENTRATED WASH BUFFER	25 ml
FATG BUFFER	30 ml
FABG BUFFER	40 ml
W 1 WASH BUFFER	45 ml
LYSIS BUFFER	135ml

2.4.2 The Primer Preparation

The primer utilized in this investigation was supplied in lyophilized form. To make a stock solution with a concentration of 100 pmol per ul, dissolved in sterile (ddH2O) (Table 3). Prior to being utilized in the PCR amplification procedure, a functional primer suspension was made by mixing 10 ul of a previous stock solution with 90 µL of sterile ddH2O. Subsequently, the extracted DNA, forward and reverse primers, and the mixture content table are combined to create the PCR master mix.

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Table 3: Primer utilized in this study

Primer	Sequence	Primer sequence 5'- - 3'	Tm(°C)	GC%	
RmpA	F	ACTGGGCTACCTCTGCTTCA	63.1	55	516
	R	CTTGCATGAGCCATCTTTCA	57.1	45	

2.4.3 Reaction mixture for PCR

followed the preparation of the master mix of the PCR by combining the extracted DNA, forward and reverse primers, and the mixture content table. The master mixture content list in Table 4 and the ideal conditions for the gene rmpA (virulent gene) employed in the current study are shown in the listed table5.

Table 4: PCR reaction components

Constituents	Volume
PCR reaction mix	12.5µl
Sense primer	10 picomols/µl (1 µl)
Antisense primer	10 picomols/µl (1 µl)
Distill water	9µl
Template DNA	1.5µl

Table 5:The ideal state for rmpA(regulator of mucoid phenotype A detecting)

STEP	Temperature	Duration	Cycles count
Initial Denaturation	95°C	4 min	1 cycle
Denaturation -2	95°C	30 Sec	35 cycle
Annealing	58°C	1 min	
Extension-1	72°C	45 Sec	
Extension -2	72°C	7 min.	1 cycle

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2.4.4. Gel Electrophoresis protocol

For the agarose gel preparation, one gram of the powder was used. It was put into a flask that measured 100 milliliters with 1 XTAE solution, 100 milliliters of it thoroughly mixed, and then heated in an oven block until the agarose was dissolved and the mixture became clear. Followed by adding the dye, the ends of the casting tray were then sealed with two taps to stop any leaks. The tines were then carefully positioned in the casting tray to create wells for loading samples, and the melted agarose was subsequently added to the tray. To demonstrate the presence of these target genes that were indicated under UV-trans illuminator, a positive result indicating the presence of the genes in the samples, when clear bands of DNA were generated at their molecular size, and the results of loading samples.

2.5 Statistical Analysis

The results were analyzed using SPSS (the Statistical Package for the Social Sciences) version 22. And the association between virulence traits was tested by the chi-square test, with a value ($p< 0.05$) considered significant.

3. Result

3.1. Bacterial Isolates Collection:

During a period of two months,75 *Klebsiella pneumoniae* clinical isolates were gathered from different sources. Each sample was labeled with the patient's details and collected using a transportation medium. Urine-harmful Klebsiella strains, the next most prevalent kidney infection, are among the most common causes of bladder infections, especially in the clinical context [12]. Biological laboratories of the Department of Science in (Mustansiriyah) served as the study's settings. Among the detected samples, more than half were isolated from urine specimens, accounting for 44 out of 75 (58.6%), demonstrating a high dissemination of infections of the urinary system, notably in those in hospitals. Eighteen out of 75, or 24% of the remaining isolates, came from wound samples, whereas the rest, or 4%, came mostly from burn victims (Figure 1). The fewest came from a stool sample (two percent),

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while the remaining ones were found in the sputum of individuals with respiratory infections, of ten percent of the total

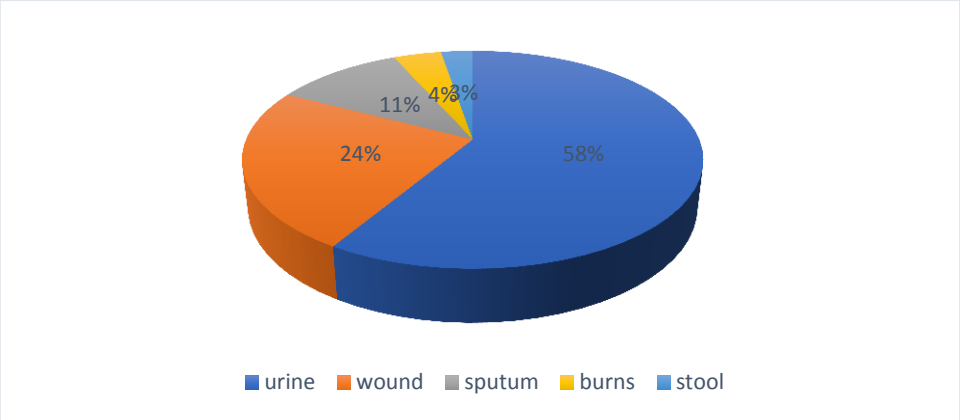

Figure 1: Percentage *of Klebsiella pneumonia* isolates from various sources

3.2 Phenotypic identification of the bacterial isolates

When cultured on various media, such as the one that is a differential and selective medium that is commonly utilized to identify and isolate gram-negative organisms, following the incubation of the cultivated plate, the colonies appear as big, pink, due to the fermentation of lactose, with a mucus texture, as these were the main and general characteristics of *the k.pneumoniae.* Additionally, the bacteria had been grown on Eosin methylene blue, which is a common differential medium. As soon as incubation, the colonies appear mucus-like, smooth, with a purple colour, unlike the other common enteric bacteria, Escherichia, which forms a green metallic sheen upon culturing on EMB(Figure 2).

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Figure 2*: Klebsiella pneumonia* colonies on MacConkey agar plates and Eosin methylene blue agar

3.2.1. Results of the biochemical analysis

The details of biochemical tests, the system of analytical profile index, which was performed, and the results recorded, show an excellent result for the identification of isolates with a high percent 97% figure 3. A seven-character analysis number that confirms the confirmed existence of *Klebsiella pneumoniae* 97% of the time is displayed by the API-20E test based on both positive and negative results, for every biochemical test that was carried out. Additionally, semi-solid media that were stabbed with bacterial culture were used to assess the motility of bacterial isolates. The results of the incubation process indicated that the bacteria were not motile because neither turbidity nor growth appeared away from the stab.

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Figure 3: The API 20E system for *Klebsiella pneumoniae* identification

3.3. Antibiotic susceptibility test

The results of a susceptibility test that was conducted on each isolate using the Kirby-Bauer technique on Miller-Hinton agar, figure 4, which involved using 13 antibiotic discs. Among the beta-lactam groups, such as penicillins, cephalosporins, and monobactams, the highest resistance rates were displayed among the tested isolates in this study, which presents the resistance rate to each drug. Penicillin G and 65 isolates show high resistance (86.7%). Amoxicillin also exhibited a greater pattern of resistance, with 60 isolates (80.0%) against the medication, the resistant rate by the isolate reached 80% percent to commomly applied drugs, including oxacilline, pencilline, other show 60% of resistant to Rifampicin, cephalosporin including,(ceftazidim, cefepime)both shown moderate resistant about 40 isolates (53.3%) are resistant and number of 15 isolates (20.0%) were intermediate, and 26 .7of strains were susceptible. drugs were found to be highly susceptible and effective: carbapenems among this broad specturm drug, including Dorepenem, had

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the highest sensitivity rate that reached (82.7%), as well. Additionally, colistin had the greatest level of sensitivity rate of ninety percent and is thought to be the most effective medication in the current study, Table 6.

Figure 4 Antibiotic sensitivity test for Klebsiella pneumonia Ciprofloxacin, Doripenem, Ceftazidime, Gentamicin

Table 6: the antibiotic discs and percentage of the isolates that showed(resistant, sensitive, and intermediate),

NO.	Antibiotic	Sensetivity%	Intermediate%	Resistant%
1	Ciprofloxacin	28(37.2%)	10(13.4%)	37(49.2%)
2	Oxacillin	7(9.3%)	3(4.3%)	65 (86.7%)
3	Rifampicin	11(14.6%)	7(9.2%)	57 (76.0 %)
5	Ofloxacin	27 (36.0%)	8(10.6%)	40 (53.3%)
6	Aztreonam	25(33.3%)	6(8.0%)	44(58.7%)
7	Amoxicillin	10(13.3%)	5(6.7%)	60 (80.0%)
8	Penicillin G	5(6.7%)	3(4.0%)	67(89.3%)
9	Vancomycin	9 (12.0 %)	5(6.7%)	60(80.0%)
10	Doxycycline	36(48.0%)	11(14.6%)	28(37.3%)
11	Gentamicin	44(58.7%)	8(11.0%)	23(30.6%)
12	Cefepime	28.(37.3%)	7(9.3%)	40(53.3%)
13	Ampicillin/sulbactam	22(29.3%)	10(13.3%)	43(57.3%)

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3.4 Phenotypic Biofilm Detection

In the current study It was demonstrated, that out of atotal of 75 clinical isolates that were tested for their capacity .to develop of biofilm through the use of method of (microtiter plate assay) figure 5, it was shown that the majority of clinical isolates were biofilm producer the total number estimated (86.6%), which classify to the following: among these percentage, a number of (26.7%) isolates, were observed to form a strong boifilm, and 37.3% were a moderate producer to biofilm, while weak producer were detected in 21.3% isolates which indicates their limit abiliity to form structured communities, and non producer were 14.7%. Using a plate assay, the test results were measured at 570 nm.

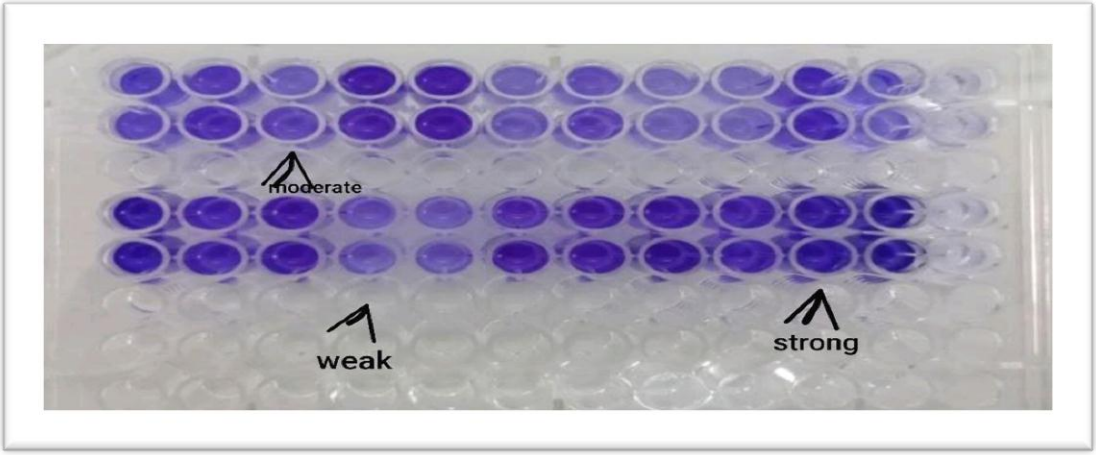

Figure 5: Detection of biofilm formation(microtiter plate assay method)

3.5. Molecular analysis for Klebsiella pnuomonia isolates

The present study employed a molecular diagnosis procedure to confirm the presence of a specific gene among the collected clinical isolates that cannot be detected by biological phenotype testing alone. The target gene (virulence gene that induce bacteria pathogenecity(rmpA), Regulator of mucoid phenotype A), was amplified using the multiplex PCR analysis method, which was followed by gel electrophoresis of *the rmpA*(regulator of mucoid phenotype)gene from *k. Pneumonia* isolates were confirmed, as it was separated on a 1% agarose gel that was dyed with red stain for

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visualization of the result of the bands at the expected product size of the PCR product *(rmpA* size 516bp) under the UV transilluminator. For the detection of the virulence gene, *rmpA* showed a high prevalence among the selected isolates, as it has been reported in 44/75 isolates (58.6%), following gel electrophoresis, present of the DNA bands indicate positive result as it shows at 516bp clear positive bands appeared at the expected size of the tested pcr product (figure 6) . as it has shown apositively association between the presence of *rmpA*, and mucoviscosity phenotypic (indicate prevalence of virulent strains) that has a major role in the regulation of capsule synthesis. Gel electrophoresis of the *rmpA*(regulator of mucoid phenotype)gene from k. Pneumonia isolates were confirmed.

Figure 6 shows the results of gel electrophoresis of the gene *rmpA*

The current study examined the correlation between biofilm formation, antibiotic resistance, and the presence of the virulence gene (*rmpA*) among *k. pneumoniae* isolates. It was demonstrated that antibiotic resistance increased with the biofilm formation strength, where the strong and moderate producers exhibited higher resistance rates in contrast to weak and non-biofilm producers, according to the demonstrated results,

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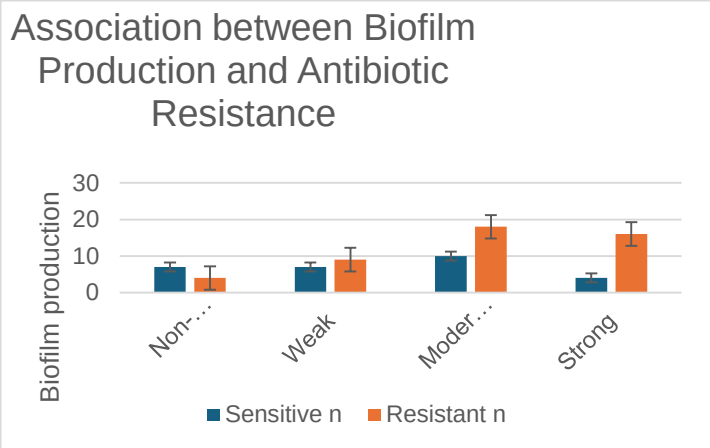
data were analyzed, and a strong correlation was indicated between the capacity to develop biofilm, among isolates and antibiotic resistance, as the strong biofilm producers exhibited a high resistance rate (80.0%), while non-producers displayed the lowest resistance rates (36.4%). Statistical analysis, although using one-way ANOVA, showed no significant differences between antibiotic resistance and biofilm development (P-value 0.218), Table 7. This finding indicates that biofilm development could enhance bacterial survival against medications. Regarding the association between rmpA and this complex matrix, a positive association was observed in Table 8, as the isolates that carry the gene 44/75 formed a strong biofilm. Among the biofilm-forming isolates, the majority carried the gene, compared to non-producing isolates. Although this relationship was strain-dependent.

Table 7: Association between Biofilm Production and Antibiotic Resistance With graphical representation



Biofilm production	Sensitive n	Resistant n
Non-producer	7	4
Weak	7	9
Moderate	10	18
Strong	4	16

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Anova: Single Factor

SUMMARY

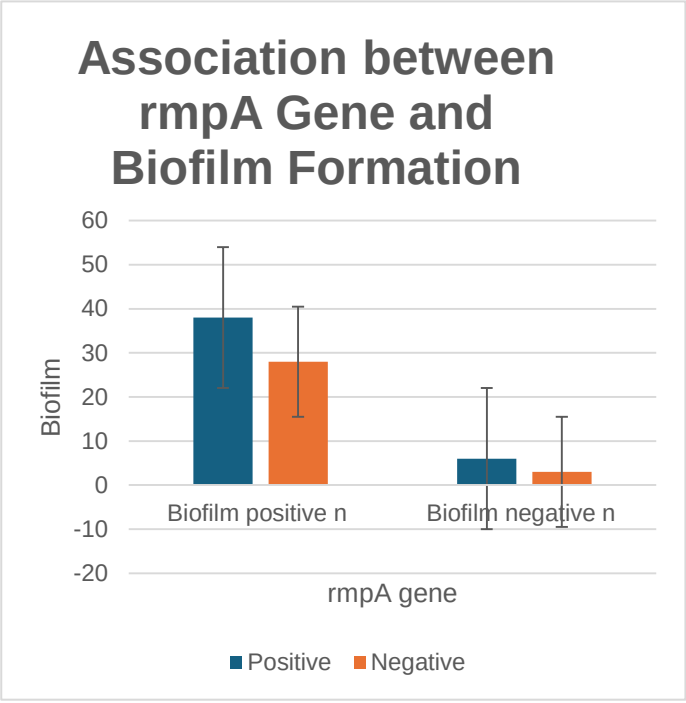
Groups	*Count*	*Sum*	*Average*	*Variance*
Sensitive n	4	28	7	6
Resistant n	4	47	11.75	41.58333

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	45.125	1	45.125	1.896673	0.217623109	5.987378
Within Groups	142.75	6	23.79167			
Total	187.875	7				

Table 8 Association between rmpA Gene and Biofilm Formation, with graphical representation

Positive	38	6	44
Negative	28	3	31
Total	66	9	75



Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Biofilm positive n	3	132	44	388
Biofilm negative n	3	18	6	9

ANOVA

Source of	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*

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Variation						
Between Groups	2166	1	2166	10.91184	0.029841	7.708647
Within Groups	794	4	198.5			
Total	2960	5				

4. Discussion

K. pneumoniae is the most important clinical pathogen that has caused the general public to become extremely concerned. According to the study by [12,13]. Urinary pathogenic Klebsiella species are the most common cause of urinary tract infections in both community and clinic settings. These species are efficient urinary system infections because they possess the traits of virulence needed to live on and adhere to the urine epithelium, cause tissue damage, and evade host immune responses. Based on current reports. *K. pneumoniae,* an Enterobacteriaceae that is widespread around the world, is one of the common infections that causes a variety of ailments and shows signs of developing antibiotic resistance. As it was shown from the results of the AST test, were the highest percentage of the collected isolates, reached 70 -80 % of resistance to classes of drugs in particular to beta lactams, which considedred the commonly applied drugs in hospitals, However, the broad spectrum antibiotics (carbapenems) were often the most effective against *K. pneumoniae* isolates, with high susceptibility seen against other antibiotics, notably DORIPENE, (82.0%). The high resistance rate that was shown agains betalactams were similar to other recent study states by [14], where the resistance to beta-lactams by isolates of *K. pneumonia* reached 70-80 %. Also, the study reported by others gives the same results, such as the study by [15,16], which shows a strong resistance rate reaching 80-90% espicially against cephalosporins. Numerous factors, such as the number of samples examined, regional variations, the location of collecting the specimens, the site of infection, and the patient's genetic

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vulnerability or background, may account for this discrepancy. As described in[17], the strong resistance pattern among β-lactams was somewhat expected among these classes. *Klebsiella pneumoniae* was found to be the most common bacterial species that manufactures ESBLs. The development of drug resistance, especially to beta-lactams, is probably caused by Klebsiella pneumonia isolates producing beta-lactamase enzymes. The most common method of resistance to β-lactam medicines, which is widely distributed and exhibited by gram-negative microbes, is the production of these enzymes. It is also regarded as an important mechanism of resistance to cephalosporins and penicillins. [18]. It was shown in the current study that, among the 75 clinical isolates that were examined for their ability to form biofilm using the microtiter plate assay method, it was observed that resistant strains develop biofilms, sensitive isolates produce non-biofilms, and weak and moderate biofilms form biofilms. The current study's findings are in line with previous research, including Out of 50 *K. pneumoniae* isolates, [19], found that 40 of them manufactured biofilms. These isolates fell into three different groups: 14 /50(28%), 23/50 isolates (46%), and 3/50 (6%), which were mild, moderate, and strong creations of biofilm, respectively. The same result was demonstrated by[20], after isolation of a clinical strain, he found that out of 100 strains, 85 were the producer to biofilm. a study conducted in Iran by [21]., 93.6% of *K. pneumoniae* isolates were capable of forming biofilms, and 33% of them were able to do so significantly..the production of this virulence factor which seems to induce or enhance the capacity of isolates to cause infection .as reported in studied by [4,5], demonstrates the vital capacity of klebsiella pneumonia which has amajor influence on its pathogenecity is its capacity to develop biofilm. and it also indicates that one of the primary mechanisms of bacterial resistance is their ability to develop biofilm, making bacteria a major problem, making it hard to control bacterial diseases in humans and animals. However, the bacteria frequently develop biofilms when they are exposed to external environmental stresses, including oxidative stress, high osmotic pressure, low pH, severe

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nutritional excess or deficiency, antibiotics, and antimicrobial agents [4,5]. However, it has been observed that sensitive isolates do not develop biofilms, while resistant strains exhibit mild to moderate biofilm formation. Numerous findings have been documented in this regard. A prior study also suggested that K. pneumoniae biofilms are notorious for having a high level of antibiotic resistance [7]. For the detection of virulent genes among collected isolates, using the PCR technique, the virulent gene, rmpA, showed a high prevalence among the selected isolates (58.6%),The results ,were in line with a number of studies, including the one that reported by [22],which showed astrong association and prevalence of the mucoviscovisity related genes (including rmpA and magA) and phenotypic hypermucoviscosity,that iut of which 58 isolates with hypermucoviscosity appearance (90%), 50 isolates were positive for rmpA presence. As well, it has been reported in a recent study by [23], which also shows a highly distributed rmpA among their clinical isolates, that demonstrate rmpA gene. Aspects of mucosity were dtected to be positvely impacted the manufacture the exterior capsule, The overproduction of extracellular polysaccharide, inducing the capsule production which leads to increase the ability of pathogen in causing sever infection, as the primary puropse from the synthesis of capsule, were to help bacteria dodge the host immune system, by preventing enhanced phagocytosis during infection, [24] The mucoid phenotype of K. pneumoniae has been beneficially affected by the rmpA gene, which leads to an increase in extracellular polysaccharide synthesis., This might enhance adherence and stability within biofilm, therefore contributing to increased virulence and infection persistence. This study highlights the importance of molecular diagnosis for better understanding the mechanism of the virulent gene within this multilayer structure (biofilm). The study was limited by the number of isolates and focused only on a specific virulence gene, which restricts understanding of the full genetic basis of virulence and biofilm development.

5. Conclusion

The majority of the *Klebsiella pneumoniae* isolates formed biofilm, indicating that biofilm development is a prevalent virulence trait which incrased and enhances antibiotic resistance. A positive association was observed, where the isolates that carry the gene formed a strong biofilm capacity compared to non-producing isolates. Highlighting the role of molecular detection of additional virulence genes that enhance the stability of biofilm.which provides a better understanding of biofilm formation, which can help in the development of effective therapeutic strategies.

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كلية التربية الاساسية – الجامعة المستنصرية
P- ISSN: 3007-3650 E- ISSN: 2706-8536
Journal of the College of Basic Education Vol.32 (No.137) 2026,pp.971-995

تقييم جينrmpAكعامل ضراوة في تنظيم تكوين الغشاء الحيوي في بكتريا الكليبسيلا الرئويه

حوراء قاسم عويد (1)
قسم علوم الحياة، كليه العلم
الجامعة المستنصرية ، العراق
+9647727705379
hawra98@uomustansiriyah.edu.iq
ميسون خليفه عباس (2)
اللقب العلمي استاذ مساعد
قسم علوم الحياة، كليه العلوم
الجامعة المستنصرية ، العراق
maysoon.bio2005@uomustansiriyah.edu.iq

مستخلص البحث:
تعد بكتريا الكليبسيلا الرئويه، من العوامل الممرضه عالميا لا سيما في بيئات السريريه، حيث تسبب في انتشار عدوى خيره منها التهاب مسالك بوليه،والجهاز التنفسي،وعدوى مجرى الدم، بالاضافه إلى ارتفاع في معدلات مقاومه لأغلب المضادات المستعمله بكثره، مما يجعل العلاج بالغ الصعوبه. مما يزيد من قدره البكتريا على أحداث الأمراض، بامتلاكها العديد من عوامل الضراوه. الهدف تهدف الدراسه إلى التحري عن مقدره العزلات البكتيرية التي تم جمعها على تكوين الغشاء الحيوي،وكذلك الكشف عن جين ضراوه,rmpA وتحديد العلاقه بوجود هذا الجين وزيادهالقدره على تكوين الغشاء الحيوي. Klebsiella pneumoniaeوالتي تم جمعها من مصادر مختلفه، حيث تم تعرف عن خمس وسبعون عزله باستخدام الطرق مختبريه التقليديه. وتم أيضا اجرا فحص الحساسيه باستخدام عدد من مضادات، وكانت النتيجة ضهور نسبه عاليه من مقاومه لعدد من الادويه لا سيما مضادات تابعه إلى مجموعه بيتا لاكتام، من ضمنها اموكسيلين، فانكومايسين، و بنسيللين بنسبه وصلت إلى 80 %، وتراوحت نسبه مقاومه مجموعات أخرى ازتريونام، وسيفالوسبورينات بين 50 الى 60 %. وبالنسبه المضادات التي اضهرت كفائه عاليه منها كاربينيمات بنسبه 82 %.وفيما يتعلق باختبار تكوين biofilm, تم استخدام طريقه microtiter plate assay, حيث اضهرت النتائج اغلبيه العزلات قدرتها على تكوين الغشاء الحيوي (من متوسطه إلى قويه)، بنسبه 86.6%. وعلى المستوى الجزيئي كشفت النتائج عن توزيع متباينه للجين المستهدف في الدراسه،حيث تم الكشف عن وجود جين الضراوه rmpA بنسبه وصلت إلى 58.6 %،مع وجود علاقه قويه بتكوين العوامل المخاطيه، والتي اثبتت في العديد من الدراسات السابقه ان لها تأثير كبير على تعزيز انتاج الكبسول، مما يزيد من الالتصاق والاستقرار داخل.biofilm .

الكلمات المفتاحية: جين، الكليبسيلا الرئويه، الغشاء الحيوي.rmpA .